

WE CLAIM:

1. A method of detecting addition or removal of a phosphate group to or from a substrate comprising

5 contacting a luminescent peptide with a binding partner that binds specifically to the peptide only if the peptide is phosphorylated, or only if the peptide is not phosphorylated, wherein the peptide is a substrate for an enzyme that catalyzes addition or cleavage of a phosphate group to or from a protein, the peptide having at least one amino acid selected from the group consisting of serine and threonine, where the serine or threonine is either near a sequence that is enriched with basic amino acids, or followed in the C-terminal direction by a proline residue, and

measuring luminescence polarization from the luminescent peptide, wherein the amount of measured luminescence polarization can be related to the extent of binding between the luminescent peptide and the binding partner.

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2. The method of claim 1 further comprising the step of correlating luminescence polarization with kinase activity.

3. The method of claim 1 further comprising the step of correlating luminescence polarization with phosphatase activity.

5 4. The method of claim 1, wherein the peptide has fewer than about 15 amino acids.

5. The method of claim 1, wherein the protein and the peptide are the same.

6. The method of claim 1, wherein the protein and the peptide are different.

15 7. The method of claim 1 further comprising the step of providing at least one phosphate group on the luminescent peptide, and competing with the luminescent peptide by catalyzing formation of unlabelled phosphorylated protein.

8. The method of claim 1, wherein the binding partner binds specifically to a phosphorylated protein substantially without regard to the particular amino acid sequence of the protein.

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9. The method of claim 8, wherein the binding partner comprises a macromolecule having entrapped metal ions.

10. The method of claim 9, wherein the metal ions comprise gallium or iron.

11. The method of claim 1, wherein the substrate has serine and threonine residues.

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12. The method of claim 1, wherein the peptide is amidated on one end.

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16. The method of claim 1, wherein the peptide is selected from the group consisting of

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EKRPPSRSKYL,
EKRPSQRpSYL,
EKRPPSRpSYL,
KRREILSRRPpSYRK,
KHFPQFPpSYSAS,
pSPELERLIQC,
GSPSVRCSpSMpS,
10 RSRHSpSYAGT,
LpTPLK,
FpTPLQ,
RKRpTLRRL,
LRRApSLG,
15 KKLNRTLpSVASL,
RPRAApTF-NH₂, and
LRRApSLG-NH₂.

20 17. The method of claim 1 further comprising illuminating the sample with polarized light from a high color temperature continuous light source.

18. A method of detecting addition or removal of a phosphate group to or from a substrate comprising

contacting a luminescent peptide with a binding partner that binds specifically to a phosphorylated peptide substantially without regard to the particular amino acid sequence of the peptide, wherein the peptide is a substrate for an enzyme that catalyzes addition or cleavage of a phosphate group to or from a protein, and

measuring luminescence resonance energy transfer (FRET), and relating FRET to the extent of binding between the luminescent peptide and the binding partner.

19. The method of claim 18 further comprising the step of correlating FRET with kinase activity.

20. The method of claim 19, wherein the peptide can be phosphorylated by one or more of the following enzymes: serine/threonine kinase, threonine/tyrosine kinase, and tyrosine kinase.

21. The method of claim 18 further comprising the step of correlating FRET with phosphatase activity.

22. The method of claim 18, wherein the protein and the peptide are the same.

23. The method of claim 18, wherein the protein and the peptide are different.

24. The method of claim 18 further comprising the step of providing at least one phosphate group on the luminescent peptide, and competing with the luminescent peptide by catalyzing formation of unlabelled phosphorylated protein.

25. The method of claim 18, wherein the substrate has at least one serine or threonine residue.

26. The method of claim 18, wherein the binding partner comprises a macromolecule having entrapped metal ions.

27. The method of claim 26, wherein the metal ions comprise gallium or iron.

28. The method of claim 26, wherein the macromolecule also comprises ruthenium.

5 29. The method of claim 26, wherein the macromolecule has a molecular weight between about 20 to 30 kilodaltons.

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30. A kinase substrate comprising
a luminophore, and
a peptide sequence selected from the group consisting of

AYTGLSTRNQETYATH-NH₂,

5 YYYIE-NH₂,

GYNELNLGRREEYDVL-NH₂,

EKRPSRSKYL

EKRPSQRSYL,

EKRPSRSYL,

10 KRREILSRRPSYRK,

KHFPQFSYSAS,

SPELERLIIQC,

GSPSVRCSSMS,

RSRHSSYPAGT,

15 LTPLK,

FTPLQ,

RKRTLRL,

LRRASLG,

KKLNRTL SVASL,

20 RPRAATF-NH₂, and

LRRASLG-NH₂.

31. The substrate of claim 30 further comprising at least one phosphate substituent that can be cleaved by a phosphatase enzyme.

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32. The substrate of claim 30, wherein the substrate can be phosphorylated by tyrosine kinase, the peptide having an amino acid sequence selected from the group consisting of

AYTGLSTRNQETYATH-NH₂,

YYYIE-NH₂, and

GYNELNLGRREEYDVL-NH₂.

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33. The substrate of claim 30, wherein the substrate can be phosphorylated by a serine/threonine kinase, the peptide being selected from the group consisting of

EKRPSRSKYL,

EKRPSQRSYL,

5 EKRPSRSYL,

KRREILSRRPSYRK,

KHFPQFSYSAS,

SPELERLIIQC,

GSPSVRCSSMS,

10 RSRHSSYPAGT,

LTPLK,

FTPLQ,

RKRTLRL,

LRRASLG,

15 KKLNRRTLSVASL,

RPRAATF-NH₂, and

LRRASLG-NH₂.

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group

34. A composition comprising at least one peptide selected from the following

EKR_PSRSKYL,

EKR_PSQR_PSYL,

EKR_PSR_PSYL,

KRREILSRR_PSYRK,

KHF_PQF_PSYSAS,

pSPELERLIIQC,

GSPSVRC_PSM_PS,

RSRHS_PSYPAQT,

L_PTPLK,

F_PTPLQ,

RKR_PTLRRL,

LRR_PSLG,

KKLNRTL_PSVASL,

RPRAA_PTF-NH₂, and

LRR_PSLG-NH₂.

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35. The composition of claim 34 further comprising a luminophore associated with at least one of the peptides.

5 36. A composition comprising a binding partner that binds specifically to a peptide having at least one serine or threonine, where the serine or threonine is either near a sequence that is enriched with basic amino acids, or followed in the C-terminal direction by a proline residue.

10 37. The composition of claim 36, wherein at least one of said serine or threonine is phosphorylated.

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38. The composition of claim 36, the binding partner binds specifically to at least one peptide selected from the following group

EKRPPSRSKYL,

EKRPSQRPSYL,

EKRPPSRPSYL,

KRREILSRRPPSYRK,

KHFPOQFP SYSAS,

pSPELERLIIQC,

GSPSVRCSpSMpS,

RSRHSpSYAGT,

LpTPLK,

FpTPLQ,

RKRpTLRRL,

LRRApSLG,

KKLNRTLpSVASL,

RPRAApTF-NH₂, and

LRRApSLG-NH₂.

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39. The composition of claim 36 further comprising a luminophore associated with at least one of the specific binding partners.

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40. A compound of the formula

FLApYTGLSTRNQETpYATH-NH₂,

FLpYpYpYE-NH₂, or

FLGpYNELNLGRREEpYDVL-NH₂,

wherein FL represents a luminophore and p represents a phosphate group.

41. The compound of claim 40, wherein said luminophore is fluorescein or a fluorescein derivative.

44. A method for determining tyrosine kinase activity, the method comprising:
performing a tyrosine kinase reaction in a solution to produce a phosphorylated
product,

5 adding a mixture of stop solution, anti-phosphotyrosine antibody, and
luminescently labeled tracer, wherein the stop solution stops the kinase reaction and the
luminescently labeled tracer competes with the phosphorylated product for binding to the
anti-phosphotyrosine antibody, and
measuring the luminescence polarization of the solution.

10 45. A method as in claim 44 conducted in a microplate well.

46. A method as in claim 44, wherein the stop solution comprises a chelator.